

## UPTAKE AND ASSIMILATION OF AMINO ACIDS BY *PLATYMONAS*. II. INCREASED UPTAKE IN NITROGEN-DEFICIENT CELLS<sup>1</sup>

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Nitrate, ammonia, and atmospheric nitrogen are usually considered the only significant nitrogen sources for marine phytoplankters (Dugdale and Goering, 1967). However, organic nitrogen as free amino acid is present in nearshore and estuarine waters at concentrations comparable to those of nitrate and ammonia.  $5 \times 10^{-7}$  to  $5 \times 10^{-6}$  moles/liter of total dissolved amino acids have been reported by several investigators (Chau and Riley, 1966; M. E. Clark, California State College at San Diego, personal communication; Degens, 1968; Hobbie, Crawford and Webb, 1968; Webb and Wood, 1967).

North and Stephens (1967, 1969) have studied the uptake and assimilation of glycine by *Platymonas*, a rock pool phytoplankter which is occasionally abundant in inshore locations. This organism grows rapidly in batch culture with glycine, nitrate, or ammonia as its sole nitrogen source (supplied at  $2 \times 10^{-3}$  moles/liter). Cells harvested from such cultures are capable of rapid and continuous uptake of glycine- $C^{14}$  present at low, natural concentrations ( $1 \times 10^{-6}$  moles/liter). Uptake rates are proportional to concentration in this concentration range. The labelled amino acid in the cells enters oxidative and synthetic pathways. The contribution of amino acid nitrogen accumulated from low ambient concentration to the nitrogen requirement of the cells can be evaluated from measurements of growth rate, amino acid uptake velocity, and cell nitrogen.

This report describes the stimulation of amino acid uptake in *Platymonas* grown on restricted nitrogen supply. The possibility of interactions between amino acid uptake and the acquisition of other nitrogenous compounds in the environment is also discussed. For example, nitrate reductase formation is suppressed by ambient ammonia in some algae (Epply, Coatsworth, and Solórzano, 1969). Conversely, amino acids are known to suppress and desuppress nitrate reductase synthesis in some higher plants (Filner, 1966). Finally, data concerning uptake of dicarboxylic and polybasic amino acids and their suitability as a nitrogen source are presented.

### MATERIALS AND METHODS

The original culture of *Platymonas*, the organism employed, was obtained from H. A. Lowenstam (California Institute of Technology). The culture medium contained  $2 \times 10^{-3}$  or  $2 \times 10^{-4}$  g-at N/liter (as nitrate, glycine, glutamate, or arginine),  $2 \times 10^{-4}$  moles/liter  $K_2HPO_4$ ,  $10^{-5}$  moles/liter  $FeCl_3$ , and 0.001% EDTA in artificial seawater (Instant Ocean, Aquarium Systems Inc., Ohio). Sterile liquid cultures were inoculated from axenic agar slants. Cultures were maintained at 20° C with cool-white fluorescent lights ( $10^4$  ergs/cm<sup>2</sup>/sec). Con-

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tinuous culture apparatus as described in North and Stephens (1969) was also used for one series of experiments.

Cells grown on  $2 \times 10^{-3}$  g-at N/liter ("high" nitrogen cultures), were harvested in late log phase for uptake experiments. Cells supplied with  $2 \times 10^{-4}$  g-at N/liter ("low" nitrogen cultures) were harvested in late log or early stationary phase of the growth curve. It should be emphasized that the difference

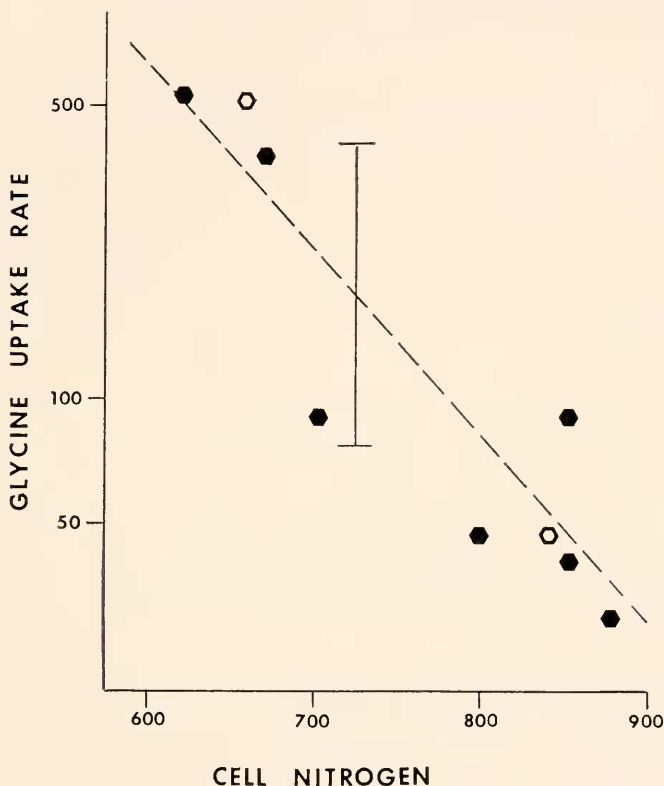


FIGURE 1. Glycine uptake *v.s.* cell nitrogen content for *Platymonas* from continuous culture. Nitrogen content varied in response to changes in dilution rate of the culture medium; abscissa:  $\mu\text{g N}/10^8$  cells; ordinate: moles glycine  $\times 10^6/\text{hr}/10^8$  cells; ( $\circ$ ) are cells from steady-state growth conditions. The vertical bar delineates 2 standard errors on each side of the regression line.

between high and low nitrogen cultures is qualitative in this work, and the initial concentration of nitrogen in both cultures is considerably higher than levels in the sea.

For amino acid uptake measurements, cells were harvested, washed, and resuspended at  $5 \times 10^5$  cells/ml in Millipore-filtered artificial seawater.  $\text{C}^{14}$  amino acids were added to give  $1 \mu\text{c}/20$  ml of cell suspension. For glycine, arginine, and glutamate, this produced substrate concentrations of 2 to  $4 \times 10^{-7}$  moles/liter. The amino acid analogues  $\alpha$ -amino isobutyric acid (AIB), 1-amino-1-carboxy-cyclo-

pentane (cycloleucine), and the amino acid taurine, had lower specific activities, and minimum substrate concentrations were about  $10^{-5}$  moles/liter (*cf.* Table II). Higher concentrations were achieved with addition of  $C^{12}$  amino acids.

To determine uptake rates, 1 ml aliquots were taken periodically. The cells in the aliquot were collected on filters ( $0.45\ \mu$ , EHWP, Millipore), washed with 10 ml artificial seawater, and the radioactivity in the cells counted. Counts/minute (cpm) in the first aliquot and the time of sampling (1 or 2 minutes) were treated as blank values and subtracted from all subsequent samples. Uptake rates were also measured by following the disappearance of  $C^{14}$  from the seawater. Radioactivity in 1 ml aliquots of seawater was counted after removing cells by filtration. All samples were counted in a Beckman CPM-100 liquid scintillation system, corrected for quenching and background, and cpm converted to moles. Amino acid uptake rates were obtained from short term observations during which rates remained linear with time.

Uptake velocities were calculated by the weighting method of Wilkinson (1961): for  $Y = Vx$  ( $Y$  = moles taken up at time  $x$  at velocity  $V$ ), the standard error (S.E.) of  $Y$  was assumed to be proportional to  $Y$  because of volumetric sampling errors. That is,  $S.E. (Y) = KY$  where  $K$  is a constant per cent error in sampling. This provides a weighting factor of  $1/Y^2$  for calculation of the velocity  $V$  and the S.E. of  $V$ .

Uptake velocities were determined at several substrate concentrations. From such data, North and Stephens (1967) have shown that glycine accumulation involves a saturable step and can be adequately described using the Michaelis-Menton equation,  $V = V_{\max}(S/S + K_s)$  where  $V$  = uptake velocity,  $V_{\max}$  = maximum uptake velocity,  $S$  = substrate concentration,  $K_s$  = substrate concentration at which the velocity is half-maximal. An unweighted  $V$  vs.  $V/S$  linear transformation of the Michaelis-Menten equation was used to evaluate the kinetic parameters  $K_s$  and  $V_{\max}$  (*cf.* Figure 2). This transformation was employed in preference to the more commonly used Lineweaver-Burk plot ( $1/V$  versus  $1/S$ ) because it provides more accurate estimates of  $K_s$  and  $V_{\max}$  (Dowd and Riggs, 1965).

Cells were extracted with cold 5% trichloroacetic acid (TCA) to provide a measure of  $C^{14}$  assimilation. This solubilizes amino acids and various small organic compounds but does not extract nucleic acids, lipids, polypeptides, or protein. In experiments with  $C^{14}$  amino acid analogues, cells were extracted with cold TCA, 80% ethanol, and 0.66 N perchloric acid. Insoluble and total radioactivity were counted directly, and soluble activity calculated by difference. Cell nitrogen content was measured with a semi-micro Kjeldahl technique (Steyermark, 1961) in continuous culture experiments and with a Coleman nitrogen analyzer for other experiments.

## RESULTS

Amino acid uptake velocities increased when nitrogen supply in the culture medium was lowered. This stimulation occurred in cells harvested from both batch and continuous culture. A logarithmic increase in rate of glycine uptake was correlated with a decrease in nitrogen content in populations from continuous culture (Fig. 1). A similar rate increase occurred in batch culture cells (Table I).

TABLE I  
Amino acid uptake by *Platymonas* grown on two concentrations of nitrate, glycine, arginine, and glutamate

| Expt | Culture conditions |                         |  | Amino acid uptake          |   |   |   | Nitrogen accumulation                                       |                       |
|------|--------------------|-------------------------|--|----------------------------|---|---|---|---|-----------------------|
|      | Nitrogen source    | Nitrogen concentration* | Cell number<br>( $\frac{\text{cells} \times 10^{-6}}{\text{ml}}$ ) | C <sup>14</sup> amino acid | Amino acid concentration<br>(moles/liter) | Uptake rate<br>( $\frac{\text{moles} \times 10^9}{\text{hr } 10^8 \text{ cells}}$ ) | Uptake rate†<br>( $\frac{\mu\text{g N}}{\text{hr } 10^8 \text{ cells}}$ ) | N content<br>( $\frac{\mu\text{g N}}{10^8 \text{ cells}}$ ) | Gen. Time‡<br>(hours) |
| 1    | NO <sub>3</sub>    | low                     | 7.9  | glycine                    | $3.6 \times 10^{-7}$                      | 128   | 2.42  | 562   | 160                   |
|      | NO <sub>3</sub>    | high                    | 9.1  | glycine                    | $3.6 \times 10^{-7}$                      | 32  | 0.60  | 870   | 1005                  |
|      | GLN                | low                     | 15.2   | glycine                    | $3.6 \times 10^{-7}$                      | 60  | 1.13  | 382   | 234                   |
|      | GLU                | high                    | 14.8   | glycine                    | $3.6 \times 10^{-7}$                      | 4   | 0.07  | 838   | 8290                  |
| 2    | NO <sub>3</sub>    | low                     | 9.3  | glycine                    | $4.2 \times 10^{-7}$                      | 152   | 2.54  | 379   | 103                   |
|      | NO <sub>3</sub>    | high                    | 20.7   | glycine                    | $4.2 \times 10^{-7}$                      | 39  | 0.64  | 474   | 514                   |
|      | ARG                | low                     | 8.8  | glycine                    | $4.2 \times 10^{-7}$                      | 215   | 3.43  | 563   | 114                   |
|      | ARG                | high                    | 26.2   | glycine                    | $4.2 \times 10^{-7}$                      | 43  | 0.72  | 815   | 785                   |
|      | GLU                | low                     | 9.0  | glycine                    | $4.2 \times 10^{-7}$                      | 183   | 3.06  | 473   | 107                   |
|      | GLU                | high                    | 40.8   | glycine                    | $4.2 \times 10^{-7}$                      | 99  | 1.65  | 513   | 215                   |
|      | NO <sub>3</sub>    | low                     | 9.3  | arginine                   | $1.5 \times 10^{-7}$                      | 81  | 15.12   | 379   | 17                    |
|      | NO <sub>3</sub>    | high                    | 20.7   | arginine                   | $1.5 \times 10^{-7}$                      | 33  | 6.16  | 474   | 53                    |
|      | ARG                | low                     | 8.8  | arginine                   | $1.5 \times 10^{-7}$                      | 83  | 15.51   | 563   | 25                    |
|      | ARG                | high                    | 26.2   | arginine                   | $1.5 \times 10^{-7}$                      | 32  | 5.98  | 815   | 94                    |
|      | NO <sub>3</sub>    | low                     | 9.3  | glutamate                  | $2.5 \times 10^{-7}$                      | 7   | 0.18  | 379   | 1460                  |
|      | NO <sub>3</sub>    | high                    | 20.7   | glutamate                  | $2.5 \times 10^{-7}$                      | 1   | 0.02  | 474   | 19300                 |
|      | GLU                | low                     | 9.0  | glutamate                  | $2.5 \times 10^{-7}$                      | 13  | 0.37  | 473   | 886                   |
|      | GLU                | high                    | 40.8   | glutamate                  | $2.5 \times 10^{-7}$                      | 1   | 0.04  | 513   | 9120                  |

\* Low =  $2 \times 10^{-4}$  g-at N/liter; high =  $2 \times 10^{-3}$  g-at N/liter.

† Uptake rate at a substrate concentration of  $5 \times 10^{-7}$  moles/liter, calculated from measured rates assuming that rate is proportional to substrate concentration.

‡‡ Generation time for a population whose growth is limited by amino acid nitrogen uptake from  $5 \times 10^{-7}$  moles/liter:  

$$\text{Generation time} = \frac{\ln 2 \times \text{N content}}{\text{Uptake rate}}$$

The low nitrogen cells (*i.e.*, cells grown on  $2 \times 10^{-4}$  g-at N/liter) contained less cell nitrogen and acquired amino acids from solution more rapidly than high nitrogen cells (grown on  $2 \times 10^{-3}$  g-at N/liter). Stimulation also ap-

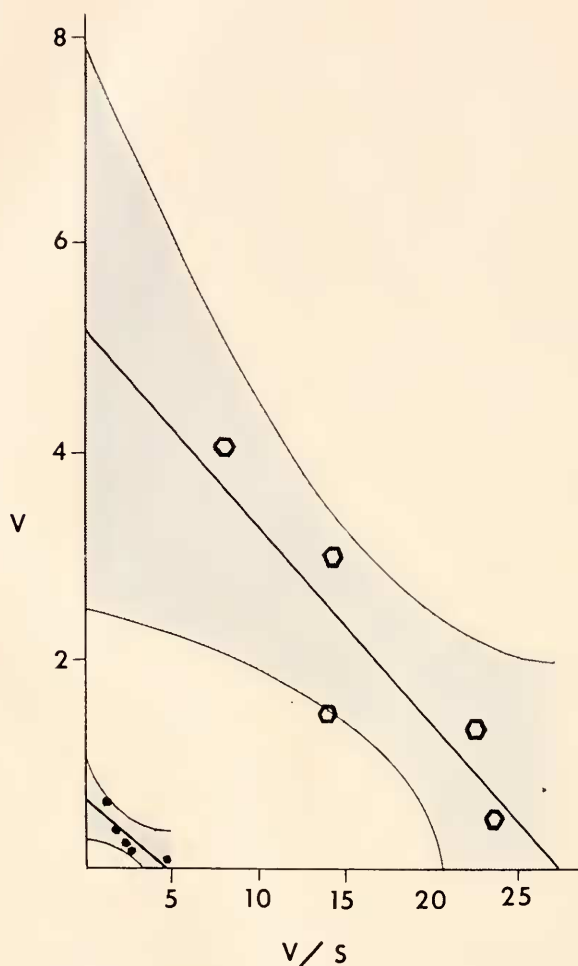


FIGURE 2. The relation between glycine uptake rates and glycine concentration for cells grown on  $2 \times 10^{-3}$  and  $2 \times 10^{-4}$  M  $\text{NO}_3^-$ . The plot is a linear transformation of the Michaelis-Menten equation:  $V = K_s(V/S) + V_{\max}$ ; abscissa:  $V/S$  where  $V$  = moles glycine  $\times 10^6/\text{hr}/10^8$  cells and  $S$  = moles glycine  $\times 10^7/\text{liter}$ ; ordinate:  $V$  = moles glycine  $\times 10^6/\text{hr}/10^8$  cells. The solid lines were determined by a least squares analysis. The dotted line encloses the 95% confidence band.

peared to be independent of the form in which the nitrogen was supplied during growth. Four different nitrogen sources were employed (nitrate, arginine, glutamate, and glycine) each at high and low concentrations. Division rates in log phase were comparable on all substrates and at both nitrogen concentrations.

Cells from each culture were checked for their ability to acquire arginine, glycine, and glutamate from dilute solution. The resulting uptake patterns were comparable in each high and low nitrogen pair (Table I). In every case, rates from low nitrogen cultures were substantially higher than corresponding high nitrogen cells. In addition, arginine uptake always exceeded glycine, and glutamate rates were always lowest. This pattern was independent of the nitrogen source on which the cells had been grown. Even though low nitrogen conditions consistently increased amino acid uptake rates and decreased cell nitrogen, the overall correlation between nitrogen content and uptake rate found in continuous culture (Figure 1) was not found in these batch culture experiments.

In the first experiment summarized in Table I, cells were harvested in log phase

TABLE II  
*Uptake of amino acids and analogues by Platymonas grown  
on two concentrations of nitrate*

| Culture conditions                  |                        | Analogue uptake          |                                |   |                 | Accumulation ratios **                      |
|-------------------------------------|------------------------|--------------------------|--------------------------------|---|-----------------|---|
| [NO <sub>3</sub> ]<br>(moles/liter) | [Cell]<br>(cells/ml)   | C <sup>14</sup> analogue | Concentration<br>(moles/liter) | Uptake<br>rate<br>(moles × 10 <sup>9</sup><br>hr 10 <sup>8</sup> cells) | Uptake*<br>rate | (moles/liter inside<br>moles/liter outside) |
| 2 × 10 <sup>-4</sup>                | 5.9 × 10 <sup>5</sup>  | GLYCINE                  | 4.7 × 10 <sup>-7</sup>         | 194   | 4128            | —   |
| 2 × 10 <sup>-4</sup>                | 5.9 × 10 <sup>5</sup>  | AIB                      | 114 × 10 <sup>-7</sup>         | 372   | 326             | 254   |
| 2 × 10 <sup>-4</sup>                | 5.9 × 10 <sup>5</sup>  | CYCLOLEUCINE             | 127 × 10 <sup>-7</sup>         | 112   | 88              | 65  |
| 2 × 10 <sup>-4</sup>                | 5.9 × 10 <sup>5</sup>  | TAURINE                  | 218 × 10 <sup>-7</sup>         | —   | —               | —   |
| 2 × 10 <sup>-3</sup>                | 16.8 × 10 <sup>5</sup> | GLYCINE                  | 4.7 × 10 <sup>-7</sup>         | 25  | 532             | —   |
| 2 × 10 <sup>-3</sup>                | 16.8 × 10 <sup>5</sup> | AIB                      | 114 × 10 <sup>-7</sup>         | 26  | 23              | 18  |
| 2 × 10 <sup>-3</sup>                | 16.8 × 10 <sup>5</sup> | CYCLOLEUCINE             | 127 × 10 <sup>-7</sup>         | —   | —               | —   |
| 2 × 10 <sup>-3</sup>                | 16.8 × 10 <sup>5</sup> | TAURINE                  | 218 × 10 <sup>-7</sup>         | —   | —               | —   |

\* Uptake rate at a substrate concentration of 10<sup>-5</sup> moles/liter, calculated from measured rates assuming that rate is proportional to substrate concentration.

\*\* Inside concentration calculated using packed cell volume (70 × 10<sup>-14</sup> liters H<sub>2</sub>O/cell).

growth when cell densities and growth rates were comparable in both high and low nitrogen cultures. Therefore, nitrogen was not growth-limiting in this experiment. In the second experiment, the low nitrogen cultures were in the early stationary phase, were less dense than the high nitrogen cultures, and presumably had exhausted the available nitrogen. Nonetheless, amino acid uptake was stimulated in both experiments.

The maximum velocity of glycine uptake ( $V_{\max}$ ) and the concentration at which the system mediating uptake was half-saturated ( $K_s$ ) were evaluated for high and low nitrogen cells. Glycine uptake rates were measured at ambient concentrations ranging from 2 × 10<sup>-6</sup> to 5 × 10<sup>-5</sup> moles/liter for low and high nitrogen cultures (Figure 2).  $K_s$  and  $V_{\max}$  were evaluated from the slope and intercept of the line. In cells from low nitrogen cultures,  $V_{\max} = 5181 \pm 2677$  ([moles glycine × 10<sup>-9</sup>/hr 10<sup>8</sup> cells] ± 95% confidence interval),  $K_s = 19 \pm 15$  ([moles glycine × 10<sup>-6</sup>/liter] ± 95% confidence interval). In high nitrogen cultures,  $V_{\max} = 644 \pm 381$ ,  $K_s = 14 \pm 14$ . A portion of the increase in  $V_{\max}$  may be due to the larger size of cells from the low nitrogen culture. These cells may

have 50% more surface area than high nitrogen cells. However, this explanation accounts for only a fraction of the difference observed in  $V_{max}$ .

Thus, amino acids were acquired from the medium more rapidly by cells grown on restricted nitrogen, whether in continuous culture or in fixed volume cultures. It was possible that stimulation of uptake resulted from more rapid utilization of amino acid within the cells (*i.e.*, assimilation), particularly since glycine is rapidly converted into an array of alcohol-soluble compounds (North and Stephens, 1967). This could stimulate uptake rates without enhancing the ability of the cell to accumulate amino acids against an internal concentration gradient. Accumulation of

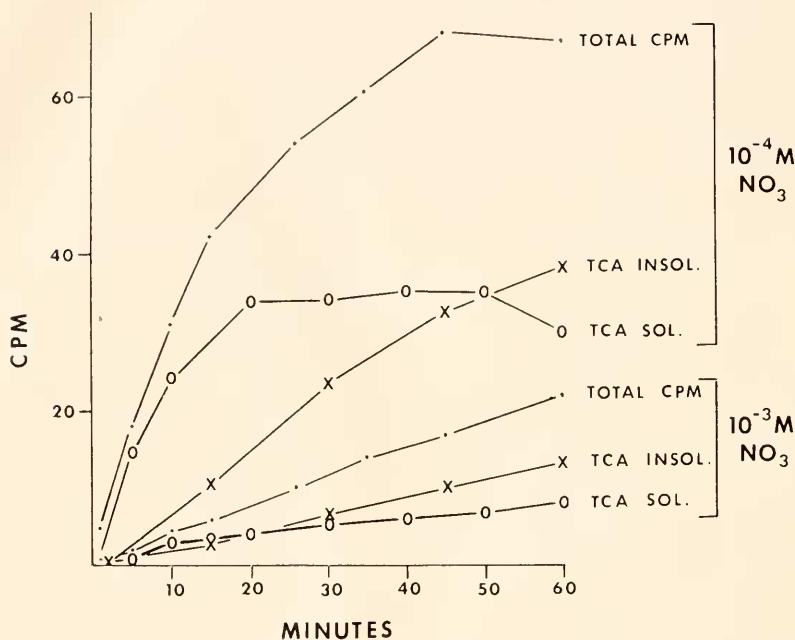


FIGURE 3. Incorporation of glycine- $C^{14}$  into TCA-soluble and TCA-insoluble fractions of *Platymonas* grown on  $2 \times 10^{-3}$  and  $2 \times 10^{-4} M NO_3$ ; (·—·) represents total CPM in cells; (X—X) CPM in TCA-insoluble fraction; (O—O) CPM in TCA-soluble fraction.

$C^{14}$  taurine and  $C^{14}$  amino acid analogues by cells grown in high nitrogen and low nitrogen batch cultures was compared. Taurine was not acquired by the cell under any of the conditions employed. Alpha-aminoisobutyric acid (AIB) and 1-amino-1-carboxy-cyclopentane (cycloleucine), however, are accumulated much more rapidly by nitrogen deficient cells (Table II). AIB- $C^{14}$  and cycloleucine- $C^{14}$  in the cells were readily extracted with 80% ethanol, verifying that these analogues were not metabolized into insoluble compounds. In another experiment, AIB was extracted quantitatively from *Platymonas* and identified by autoradiography of a thin layer chromatogram of an extract (perchloric acid). The ratio of internal to external concentration of these analogues was high at the end of the experiment (Table II) and indicated accumulation against a substantial concentration differ-

ence. Larger ratios would have been achieved had the experiment continued since uptake was still linear at the end of the observations. Thus, uptake stimulation is accompanied by increased rates of accumulation into the cell.

The rate at which glycine radioactivity was assimilated into the cold TCA-insoluble fraction of cells from low and high nitrogen cultures was also investigated. The amino acid  $C^{14}$  obtained from the medium entered synthetic pathways more rapidly in the low nitrogen cells (Fig. 3). No conclusions can be drawn about absolute rates of amino acid assimilation without further information about the size and specific activities of amino acid pools. However, the data indicate that ambient amino acids were incorporated into cells of either history.

The rates of arginine uptake were unaffected by the presence of nitrate or ammonia at levels 20 to 1000 times more concentrated than the amino acids (Table III). A similar result was obtained with glycine- $C^{14}$ . Thus, there is no short term interaction at levels of inorganic nitrogen as high as  $10^{-4}$  moles/liter.

TABLE III

*Arginine uptake by Platymonas in the presence of nitrate and ammonia. Cells were incubated in  $1.5 \times 10^{-7}$  M arginine- $C^{14}$  for 10 minutes*

|                    | % radioactivity remaining in medium at 10 minutes |                      |
|--------------------|---|----------------------|
|                    | High nitrogen culture                             | Low nitrogen culture |
| control            | 75.2  | 28.6                 |
| $10^{-5}$ M $NO_3$ | 76.5  | 28.7                 |
| $10^{-4}$ M $NO_3$ | 75.5  | 29.4                 |
| $10^{-5}$ M $NH_3$ | 75.9  | 28.1                 |
| $10^{-4}$ M $NH_3$ | 78.2  | 28.7                 |

Of course, our earlier results indicate that supplying nitrate at levels around  $2 \times 10^{-3}$  moles/liter would eventually depress amino acid uptake rates, but the time course of this effect has not been investigated. However, such levels far exceed any natural concentrations that might be encountered by *Platymonas*.

## DISCUSSION

We are investigating the significance of natural dissolved amino acids to the nutrition of *Platymonas*. The fact that arginine, glutamate, and glycine can provide nitrogen for growth in batch culture is neither necessary nor sufficient to argue for a normal nutritional role of dissolved amino acids. An amino acid or other organic compound in the environment may supplement other nitrogen sources or compensate for a particular synthetic deficiency without being a complete nitrogen source. In addition, an amino acid that supports growth at high concentrations may have no importance in the sea. Nonetheless, neutral, dicarboxylic, and polybasic amino acids are all complete nitrogen sources for *Platymonas*. In general, utilization of amino acids supplied at the high concentrations characteristic of batch cultures appears to be widespread among phytoplankters (Danforth, 1962).

A more cogent approach to the nutritional role of dissolved amino acids is to study the utilization of these compounds when supplied at concentrations reported

from natural waters. The work reported here and in earlier contributions from our laboratory supports the following statements: (1.) Amino acids are accumulated by *Platymonas* from very dilute solutions, concentrations of which are in the range reported for natural waters. (2.) Accumulated amino acids enter respiratory and synthetic pathways in the cell. (3.) Uptake continues for long periods (demonstrated for glycine). (4.) The uptake rate depends on the amino acid and the nutritional history of the cells. Rates are higher in cells which have been grown under conditions where nitrogen is restricted though not to the extent that it limits the growth rate. (5.) When cells are stimulated to take up amino acids more rapidly, the pattern of uptake is constant regardless of the nitrogen source on which they were grown. The uptake rate of arginine is always greater than glycine, and glutamate is consistently lowest. (6.) The stimulation of amino acid uptake is accompanied by increased rates of accumulation, as shown by accelerated uptake of amino acid analogues. (7.) Cells grown at reduced nitrogen concentrations show an increase in the maximum velocity ( $V_{\max}$ ) of amino acid uptake. No change in  $K_s$  is apparent. (8.) Amino acid uptake proceeds in the presence of nitrate and ammonia.

The conditions that increase amino acid uptake in the laboratory might be expected to occur under natural conditions. We have previously shown (North and Stephens, 1969) that restriction of nutrient levels in continuous culture produced a marked increase in rates of amino acid uptake. In the present report, increased rates were also obtained when nitrogen supply was reduced in batch cultures. North, Stephens and North (in press) have also demonstrated comparable increases in uptake rates for various amino acids in several other marine phytoplankters.

There are other reports of amino acid uptake by phytoplankton. A light-dependent glycine transport system has been reported for the freshwater alga, *Scenedesmus* (Cseh and Szabo, 1965). Hellebust and Guillard (1967) report rapid uptake of glutamate by the diatom, *Melosira*. Glutamate uptake is also reported in a study of four phytoplankters by Sloan and Strickland (1965) though they conclude that the observed rates are too low to have any nutritional significance. In *Platymonas*, glutamate uptake is also quite slow compared to glycine and arginine. Even though growth on restricted nitrogen increases glutamate uptake ten-fold, rates are too low to supply a significant amount of nitrogen (see below). Culture conditions may be responsible for some of the differences found in the literature.

Other nitrogen-sensitive transport systems have been reported in algae. Yaden (1965) discusses increases in glucose and amino acid uptake in *Euglena*. Uptake was stimulated by heterotrophic growth in the dark, starvation in the dark, or streptomycin bleaching. Nitrate (Eppley and Thomas, 1969) and ammonia (Fitzgerald, 1968; Syrett, 1962) uptake systems may also respond to nitrogen deprivation. The former authors report an increase in  $V_{\max}$  for nitrate uptake in nitrogen-deficient cells. Although our data justify the conclusion that  $V_{\max}$  increases in batch culture cells grown under low nutrient conditions, the limitations of the batch culture technique are severe. We can neither control nor predict the growth conditions which will give reproducible values for a particular kinetic parameter in such cultures. Thus, there is no overall correlation between uptake rates and any

of the usual culture parameters such as growth rate, nitrogen content, cell concentration, *etc.* This is not surprising since the conditions in batch cultures are changing continuously and rapidly.

Even though some data reported here suffer the limitations of batch culture technique, the contribution of amino acid uptake to the nitrogen economy of the cells was evaluated. Nitrogen uptake velocities (from a solution of  $5 \times 10^{-7}$  moles/liter) were calculated for glutamate, glycine, and arginine for cells from high and low nitrogen cultures. Uptake rates, when divided by cell nitrogen content, are equivalent to growth constants in terms of nitrogen (Dugdale and Goering, 1967); that is, the constants represent growth rates or generation times (generation time =  $\ln 2/\text{growth rate}$ ) assuming that growth is limited by amino acid nitrogen. The calculated constants (Table I) indicate that glycine might supply sufficient nitrogen to support growth at generations as short as 160 hours. Arginine uptake is consistently faster than glycine, and the greater nitrogen content of the molecule could support a generation as short as 17 hours. These calculated generation times cannot be compared directly with generation times under natural conditions, since the latter have not been measured. One can speculate that natural doubling times might well be ten times longer than generation times observed in batch culture. However, if calculated generation times exceed batch culture growth by fifty-fold, the substrate concerned probably cannot contribute significantly to the economy of the organism under natural conditions. Since the shortest generation time observed in batch culture of *Platymonas* was 22 hours, we conclude that glycine and arginine may be important nitrogen sources in the sea. Uptake of glutamate was slower than both arginine and glycine and could support a generation time of 886 hours. This does not represent a significant nitrogen contribution under any of our conditions (although glutamate was a competent nitrogen source in batch culture). Again the limitations of batch culture should be emphasized.

Caperon (1969) suggests that nitrogen limited cell growth should be described by a response function which incorporates both the nutritional history of the cell and the current growth conditions. Such a response function is undoubtedly required to describe behavior of the cell populations in batch culture. Until this is done, kinetic parameters obtained from such experiments should probably not be used to predict behavior of natural phytoplankton populations. This difficulty can be eliminated if cells are grown in continuous culture and allowed to reach a steady state. Under steady-state conditions, the cells have a constant and reproducible nutritional history, and well defined growth parameters.

When generation times calculated from amino acid uptake velocities and nitrogen content are compared with measured generation times of the steady-state cell populations, a more defensible estimate of the nutritional significance of dissolved amino acids is achieved (*cf.* Figure 1). At moderate growth rates (generation time = 51 hours) and natural glycine concentrations ( $5 \times 10^{-7}$  moles/liter), the uptake system can supply sufficient nitrogen to support a calculated doubling time of 860 hours. However, at the slower growth rates achieved by reducing the nutrient supply (generation time = 166 hours), the glycine contribution becomes more important. Under these conditions, uptake rate increases and cell nitrogen decreases as in low nitrogen batch cultures. Glycine uptake could support a gen-

eration of 95 hours. This assumes that amino acid uptake continues unabated. Sustained uptake has been demonstrated for high nitrogen cells (North and Stephens, 1969), but similar experiments have not been performed on nitrogen-deficient cells.

These calculations do not correct for leakage of nitrogen-containing compounds. However, such losses are probably small. Hellebust (1965) reports that *Tetraselmis* (*Platymonas*) leaks 1–3% of its photo-assimilated carbon, depending on light conditions. If the excreted product is entirely protein, this leakage rate adds a maximum of 5% to the cell nitrogen requirement.

The amino acid molecule also provides reduced carbon to the cells. The accumulated amino acid can be expressed as a fraction of respiratory rate by calculating the oxygen required for complete oxidation of accumulated amino acid and comparing this figure to oxygen consumption in the dark. Dark respiration of *Platymonas*, as determined in a Gilson respirometer, ranges from 1.3 to 3.5  $\mu$ moles  $O_2$ /hr/ $10^8$  cells. Populations from continuous cultures can obtain 0.5  $\mu$ moles glycine/hr/ $10^8$  cells. Complete oxidation of this amount of glycine requires about 0.9  $\mu$ moles of  $O_2$ , or 25–67% of the total respiration.

This is not to say that *Platymonas* can grow exclusively on amino acid carbon in the dark, even though amino acid uptake is not light-dependent (North and Stephens, unpublished results). On the contrary, *Platymonas* appears to be an obligate phototroph (Gibor, 1956). Nonetheless, the utilization of amino acids or other organic substrates reduces the photosynthetic carbon requirements and may be important to algae in certain habitats (Bunt, 1969).

Though there is no short-term effect on amino acid uptake by high concentrations of nitrate or ammonia, we have not examined the possibility of a reciprocal situation, i.e., the effect of amino acids on the utilization of other nitrogen sources, particularly nitrate. Such investigations may be important in view of recent ecological emphasis on the characteristics of nitrate uptake. Dugdale and Goering (1967) suggest using uptake velocities for nitrate and ammonia to measure nitrogen flow in primary productivity studies *in situ*.  $K_s$  and  $V_{max}$  have been used to analyze and predict interspecies competition and succession (Dugdale, 1967; Eppley, Rogers and McCarthy, 1969). Finally, a nitrate reductase assay in the field has been proposed as a sensitive measure of rates of nitrate assimilation and the presence of nitrate (Eppley, Coatsworth, and Solórzano, 1969). Interactions between nitrite, nitrate, and ammonia assimilation were examined and taken into account in the above studies. The effect of amino acids on these systems is unknown, however, and these authors have emphasized the need for such investigations. We propose to study the effect of low concentrations of amino acids on nitrate reductase content of steady-state populations.

## SUMMARY

The marine phytoplankter, *Platymonas*, increases rates of amino acid uptake when grown on a restricted nitrogen supply. Uptake of glycine, arginine, and glutamate increases as much as 10-fold when cells are deprived of nitrogen during growth. In a given culture, arginine uptake is always faster than glycine, and

glutamate accumulation is always slowest. Nitrogen-deprivation also stimulates accumulation of non-metabolizable amino acid analogues. Kinetic studies suggest that  $V_{\max}$  increases and  $K_s$  remains unchanged in N-deprived cells. When low concentrations of glycine or arginine are supplied to cells grown on restricted nitrogen, uptake rates are sufficient to fulfill the nitrogen requirement in *Platymonas*. High concentrations of nitrate or ammonia do not interfere with amino acid uptake. Thus amino acids in the ocean may be an important nitrogen source for some phytoplankters.

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